

Rotational Dynamics of Adenine Amino Groups in a DNA Double Helix

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ABSTRACT Exocyclic amino groups of the bases undergo conformational fluctuations that affect the recognition and reactivity of nucleic acid molecules. Among these fluctuations, rotation of amino groups around C-N bonds is of special interest. In the present paper, we report the first determination of the rates and energetic parameters for rotation of the N6-amino group of adenine in a DNA double helix. The DNA molecule studied is the dodecamer $[d(CGCGAGCTCGCG)]_2$. The adenine in each A · T basepair of the dodecamer was labeled with ^{15}N at the N6 position, and the NMR resonances of the two protons in the adenine amino group were selectively observed by ^{15}N -editing methods. The rates of rotation of the amino group were obtained from experiments of transfer of magnetization between the two protons in the same group and from lineshape analysis of ^{15}N -edited amino proton NMR resonances. The results show that, over the temperature range from 0 to 70°C, the rates of rotation of adenine amino groups range from 60 to 24,000 s^{-1} . Formation of the activated state during rotation has a standard enthalpy change of 15.3 ± 0.2 kcal/mol and a standard entropy change of 6.0 ± 0.7 cal/(mol · K). Analysis of the results suggests that rotation of the amino group occurs in the paired, closed state of the adenine in the A · T basepair of the double-helical DNA structure.

INTRODUCTION

Exocyclic amino groups of adenine, guanine, and cytosine are key recognition sites in the formation of structures and in intermolecular interactions of DNA and RNA. Hydrogen bonding of amino groups maintains the specificity of canonical Watson-Crick pairing of the bases in fundamental processes such as DNA transcription and replication. Alternative structures of DNA and RNA are often built by hydrogen bonding at amino groups. For example, in TA**T* triplets, the adenine amino group is responsible for the two hydrogen bonds that anchor the three strands in the triple-helical structure. The exposure of the amino groups to the grooves of double-helical structures also makes them prime targets for binding of drugs and water molecules, and for recognition of specific base sequences by protein side chains.

Understanding the fundamental roles of amino groups in the structure and function of nucleic acids requires direct characterization of their physical, chemical, and dynamic properties. NMR spectroscopy is one technique that can provide this information. However, until recently, the majority of NMR investigations of amino groups have been limited to model systems of isolated bases or basepairs (McConnell and Seawell, 1973; Raszka, 1974; Williams et al., 1990). Spectroscopic observation of amino protons in native nucleic acid structures has been impeded by experi-

mental difficulties resulting from the overlap of their resonances with other proton resonances. In addition, resonances of amino protons are often broadened by the rotational motion of the amino group around the C-N bond. Recently, observation of amino proton NMR resonances has been greatly aided by the development of new procedures for uniform and site-specific labeling of RNA and DNA with ^{15}N (Batey et al., 1992; Jiang et al., 1997; Kelly et al., 1995; Louis et al., 1998; Mer and Chazin, 1998; Nikonowicz et al., 1992; Smith et al., 1997; Zhang et al., 1998; Zimmer and Crothers, 1995). The presence of the heteroatom allows specific editing of amino proton resonances and direct measurements of their structural and dynamic properties (Louis et al., 1998; Mueller et al., 1995; Zimmer and Crothers, 1995).

We have previously used ^{15}N and ^{15}N - ^1H relaxation measurements on ^{15}N -labeled DNA to characterize the internal motions of adenine amino groups on the subnanosecond time scale (Michalczyk et al., 1996). In the present work, we have extended this investigation of DNA dynamics to motions on the microsecond to millisecond time scale, namely, rotation of adenine amino groups around the exocyclic C-N bond. The DNA double helix investigated is the dodecamer $[d(CGCGAGCTCGCG)]_2$. Labeling of the amino nitrogens of the symmetrical adenines with ^{15}N allowed specific observation of adenine amino proton resonances and determination, for the first time, of the rate and energetic parameters for the rotation of adenine amino groups in a DNA molecule.

MATERIALS AND METHODS

Sample preparation

The DNA dodecamer was synthesized on an automated DNA synthesizer (model 381A; Applied Biosystems, Foster City, CA) using the H-phosphonate method. Adamantane carbonyl chloride was used as an activator.

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^{15}N -labeled deoxyadenosine H-phosphonate was synthesized from 6-chloropurine, using a combination of chemical and enzymatic methods, as described previously (Kelly et al., 1995; Michalczyk et al., 1996). The DNA oligonucleotide was purified by high-performance liquid chromatography (HPLC) on a PRP-1 reverse-phase column (Hamilton, Reno, NV) with a gradient of 0–12% acetonitrile in 50 mM ethylenediamine formate buffer (pH 7.0). The purified oligonucleotide was dialyzed against 0.6 M NaCl to exchange counterions and finally dialyzed for the NMR experiments into 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl and 2 mM EDTA in 90% H_2O /10% D_2O . The DNA concentration in the sample was 1.8 mM (duplex).

NMR spectroscopy

The NMR experiments were carried out in a magnetic field of 11.7 T on a Varian INOVA spectrometer or on a Bruker DMX-500 spectrometer. ^{15}N -edited proton spectra were obtained using the 1D version of the heteronuclear single-quantum coherence (HSQC) pulse sequence with water flip-back (Grzesiek and Bax, 1993). Unedited proton spectra were obtained using the WATERGATE pulse sequence (Piotto et al., 1992).

The rates of rotation of the amino groups were measured in ^{15}N -edited magnetization transfer experiments. Each adenine amino proton resonance was selectively inverted by a rectangular soft pulse (Alger and Prestegard, 1977; Michalczyk and Russu, 1997). The length of the pulse (2.5 ms) was adjusted such that the excitation of the other proton in the amino group was less than 5%. A short gradient (42 G/cm for 0.3 ms) was applied after the inversion pulse to dephase any remaining transverse magnetization. Thirty-two values of the magnetization transfer time ranging from 5×10^{-4} to 3 s were used in each experiment. Spectra were recorded using the HSQC pulse sequence with water flip-back (Grzesiek and Bax, 1993).

The rates of exchange of amino protons with water were measured in experiments of transfer of magnetization from water. The water magnetization was inverted by a 180° Gaussian pulse (10.4–10.7 ms, depending on temperature). ^{15}N -edited proton spectra were recorded at various magnetization transfer times after inversion of water resonance (14 values in each experiment ranging from 0.02 to 3 s). A weak gradient (0.21 G/cm) was applied during the magnetization transfer time to eliminate radiation damping. Relaxation of water protons after inversion was measured in a separate experiment.

The intensities and linewidths of the amino proton resonances were obtained by deconvolution, using a standard deconvolution program in Varian VNMR software.

Analysis of ^{15}N -edited magnetization transfer experiments

The magnetization transfer experiments have been analyzed by treating the amino group as an isolated two-spin system. This approximation is justified by the following facts. First, the distance between the two amino protons (1.77 Å) is much shorter than the distances between any of them and other protons in the DNA molecule (>2.5 Å for canonical B-DNA conformation). As a result, ^1H - ^1H dipolar interactions are dominated by the mutual interaction between the two amino protons, and contributions from other protons in the molecule can be neglected. Second, the contribution of the ^{15}N spin to longitudinal relaxation of amino protons is also negligible. This contribution is determined by spectral density functions $J(\omega_{\text{H}})$, $J(\omega_{\text{H}} + \omega_{\text{N}})$, and $J(\omega_{\text{H}} - \omega_{\text{N}})$, where ω_{H} and ω_{N} are the resonance frequencies of ^1H and ^{15}N , respectively. At a magnetic field of 11.7 T, $J(\omega_{\text{H}})$, $J(\omega_{\text{H}} + \omega_{\text{N}})$, and $J(\omega_{\text{H}} - \omega_{\text{N}})$ are very small because $(\omega_{\text{H}}\tau_{\text{c}})^2 \gg 1$ (e.g., correlation time τ_{c} is 7.6 ns at 5°C , and $\omega_{\text{H}}\tau_{\text{c}} = 24$; Michalczyk and Russu, 1993).

In this two-spin approximation, the dependence of the magnetization of the two amino protons (labeled A and B) on the magnetization transfer time

is described by (Ernst et al., 1987)

$$\begin{aligned}\frac{dm_{\text{A}}(t)}{dt} &= -(R_{1\text{A}} + k_{\text{r}}) \cdot m_{\text{A}}(t) + (k_{\text{r}} - \sigma) \cdot m_{\text{B}}(t) \\ \frac{dm_{\text{B}}(t)}{dt} &= +(k_{\text{r}} - \sigma) \cdot m_{\text{A}}(t) - (R_{1\text{B}} + k_{\text{r}}) \cdot m_{\text{B}}(t)\end{aligned}\quad (1)$$

where m_{A} and m_{B} are the deviations of the longitudinal magnetizations of the two protons from their equilibrium value, $R_{1\text{A}}$ and $R_{1\text{B}}$ are the longitudinal relaxation rates, σ is the cross-relaxation rate, and k_{r} is the rate of rotation of the amino group around the C-N bond. The exchange of amino protons with water is not included in Eqs. 1 because, in the experiments used, the magnetization of water protons is maintained at or close to equilibrium (Grzesiek and Bax, 1993; Michalczyk and Russu, 1997), and, as shown in the Results, the rates of exchange of amino protons with water are very small. The solutions of Eqs. 1 are

$$\begin{aligned}m_{\text{A}}(t) &= C_1 \cdot \exp(\lambda_1 t) + C_2 \cdot \exp(\lambda_2 t) \\ m_{\text{B}}(t) &= F_1 \cdot \exp(\lambda_1 t) + F_2 \cdot \exp(\lambda_2 t)\end{aligned}\quad (2)$$

where

$$\begin{aligned}\lambda_{1,2} &= -\Sigma \pm D \\ \Sigma &= \frac{R_{1\text{A}} + R_{1\text{B}}}{2} + k_{\text{r}} \\ D &= [\delta^2 + (k_{\text{r}} - \sigma)^2]^{1/2} \\ \delta &= \frac{R_{1\text{A}} - R_{1\text{B}}}{2} \\ C_{1,2} &= \frac{(D \mp \delta) \cdot m_{\text{A}}(0) \pm (k_{\text{r}} - \sigma) \cdot m_{\text{B}}(0)}{2D} \\ F_{1,2} &= \frac{\pm(k_{\text{r}} - \sigma) \cdot m_{\text{A}}(0) + (D \pm \delta) \cdot m_{\text{B}}(0)}{2D}\end{aligned}$$

These solutions can be simplified by noting that, because relaxation is dominated by ^1H - ^1H dipolar interactions within the amino group, the longitudinal relaxation rates of the two protons should be similar ($R_{1\text{A}} \approx R_{1\text{B}}$). Thus the term δ is negligible relative to $k_{\text{r}} - \sigma$, and the eigenvalues in Eqs. 2 simplify to

$$\begin{aligned}\lambda_1 &= -\frac{R_{1\text{A}} + R_{1\text{B}}}{2} - \sigma \\ \lambda_2 &= -\frac{R_{1\text{A}} + R_{1\text{B}}}{2} - 2k_{\text{r}} + \sigma\end{aligned}\quad (3)$$

It is readily seen that, for large values of the rotation rate k_{r} , $|\lambda_2| \gg |\lambda_1|$. The differences between $|\lambda_2|$ and $|\lambda_1|$ are also enhanced by the fact that, in the slow motion limit (i.e., $(\omega_{\text{H}}\tau_{\text{c}})^2 \gg 1$), the cross-relaxation rate, σ , is negative, and approaches, in absolute value, the longitudinal relaxation rate R_1 (Solomon, 1955); for example, for the two protons in the amino group at 5°C , $\sigma = -13.6 \text{ s}^{-1}$ and $R_1 = 15.1 \text{ s}^{-1}$. As a result, $|\lambda_1|$ is further decreased relative to $|\lambda_2|$.

The rate constants λ_1 and λ_2 were obtained by fitting the relaxation curves in each ^{15}N -edited magnetization transfer measurement to Eqs. 2, using the nonlinear least-squares algorithm in software Origin 4.1 (Microcal Software, Northampton, MA). To extract the rotation rate k_{r} from λ_2 , one must know the values of $(R_{1\text{A}} + R_{1\text{B}})/2$ and σ (Eqs. 3). These were calculated assuming that the dodecamer is in canonical B-DNA conformation and using the full relaxation matrix program MORASS (Post et al.,

1990). The calculations were carried out for isotropic motion and for a range of correlation times from 1 to 10 ns. The values of $(R_{1A} + R_{1B})/2$ and σ that yielded the λ_1 values determined from the nonlinear least-squares fit were selected. The use of canonical B-DNA conformation in these calculations is justified by the fact that relaxation of amino protons is dominated by dipolar interactions within the amino group, and thus it does not depend significantly on the structure of the DNA molecule. Moreover, the amino group lies in, or close to, the plane of the basepair and is perpendicular to the helix axis. In this geometry, inclusion of the anisotropy of DNA molecular tumbling does not change the spectral density functions significantly (Michalczyk, 1997; Woessner, 1962), justifying the use of an isotropic motion model in these calculations.

Lineshape analysis of ^{15}N -edited amino proton resonances

When the rotation of the amino group is slow on the chemical shift scale, the linewidths of the amino proton resonances, $\Delta\nu_{1/2}^{A,B}$, are (Sandstrom, 1982)

$$\Delta\nu_{1/2}^{A,B} = \frac{1}{\pi} \left(k_r + k_{\text{ex}} + \frac{1}{T_{2A,B}} \right) \quad (4)$$

where k_r is the rate of rotation of the amino group, k_{ex} is the rate of exchange of amino protons with water, and $1/T_{2A}^{-1}$ and $1/T_{2B}^{-1}$ are the rates of intrinsic transverse relaxation of the two amino protons. As shown in the Results, the rates of exchange of the amino protons with water, k_{ex} , are small and make negligible contributions to the observed linewidths. The intrinsic transverse relaxation rates of the two amino protons, $1/T_{2A}$ and $1/T_{2B}$, are expected to be dominated by ^1H - ^1H and ^1H - ^{15}N dipolar interactions. Any contribution from chemical shift anisotropy should be negligible because of the small anisotropies of proton chemical shifts (Lounila and Jokisaari, 1982). Moreover, the T_1 relaxation rates of the adenine amino ^{15}N nuclei in the dodecamer range from 0.2 to 0.3 s (Michalczyk et al., 1996). These values are much smaller than the ^1H - ^{15}N coupling constant ($J_{\text{HN}} = 91 \pm 1$ Hz; Michalczyk et al., 1996), thus excluding any significant contribution from scalar relaxation. The intrinsic transverse relaxation rates of the two protons were calculated, based on Eq. 4, from the observed linewidth, $\Delta\nu_{1/2}^{A,B}$, and the rotation rates, k_r , measured independently in ^{15}N -edited magnetization experiments at 2.5 and 4°C. With

increasing temperature, $1/T_{2A}$ and $1/T_{2B}$ decrease; this temperature dependence should follow the change in the correlation time of the DNA dodecamer. Assuming that the correlation time depends on temperature as η/T , as predicted by the Stokes-Einstein equation, and the viscosity of the DNA solution, η , depends on temperature, as does that of water (Weast, 1987), the correlation time should decrease from 8.1 ns at 2.5°C to 1.7 ns at 70°C. In this range $(\omega_{\text{H}}\tau_c)^2 \gg 1$, and the contribution of ^1H - ^1H dipolar interactions to transverse relaxation is dominated by the spectral density function $J(0)$. Contribution of ^1H - ^{15}N dipolar interactions is dominated by $4J(0) + 6J(\omega_{\text{N}})$ (Solomon, 1955), which, in the range of interest, also varies linearly with correlation time. Accordingly, the values of $1/T_{2A}$ and $1/T_{2B}$ at all other temperatures of interest were obtained from the values at 2.5 and 4°C and from the temperature dependence of the correlation time predicted by the Stokes-Einstein equation. The validity of this extrapolation was confirmed by measurements of the temperature dependence of the linewidths of nonexchangeable proton resonances, which were in good agreement with the temperature dependence predicted by the Stokes-Einstein equation (results not shown).

When the rotation of the amino group is fast on the chemical shift scale, a single averaged resonance is observed for the two amino protons, and, when the exchange with water is slow, the linewidth of this resonance, $\Delta\nu_{1/2}$, is (Piette and Anderson, 1959)

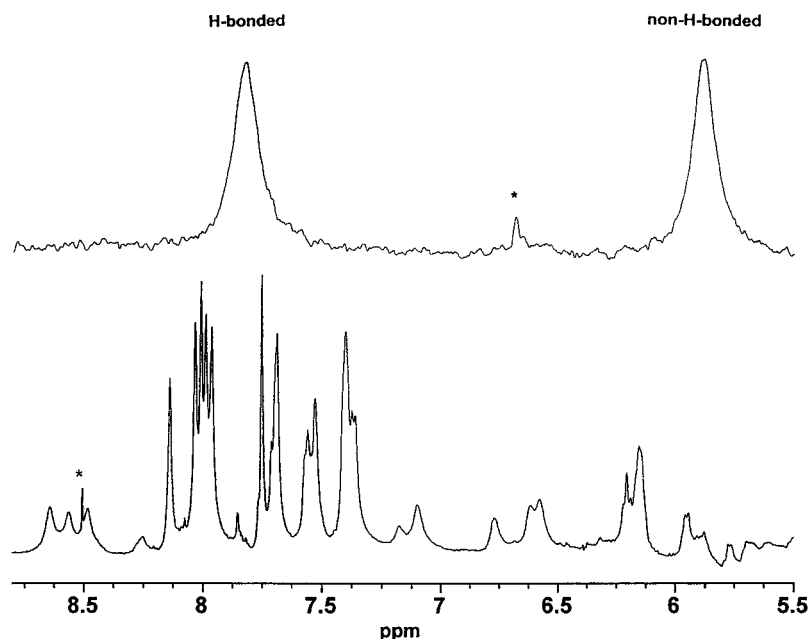
$$\Delta\nu_{1/2} = \frac{1}{2} \pi \cdot \frac{(\delta\nu)^2}{k_r} + \frac{1}{2\pi} \left(\frac{1}{T_{2A}} + \frac{1}{T_{2B}} \right) \quad (5)$$

The frequency separation between the hydrogen-bonded and non-hydrogen-bonded amino proton resonances, $\delta\nu$, was measured as a function of temperature over the temperature range corresponding to slow exchange on the chemical shift scale (i.e., from 0 to 15°C) and extrapolated linearly at higher temperatures. This linear extrapolation is justified by our previous observations that the chemical shifts of ^{15}N and nonexchangeable proton resonances in the dodecamer do not exhibit any abrupt changes in the temperature range of interest (Michalczyk et al., 1996).

RESULTS

The ^{15}N -edited proton spectrum of the dodecamer at 2.5°C is shown in Fig. 1. Because the base sequence of the

FIGURE 1 Aromatic region of the ^{15}N -edited (top) and unedited (bottom) proton spectrum of the dodecamer at 2.5°C. The vertical scale of the top spectrum is increased ~80-fold relative to that of the bottom spectrum. The resonances marked with an asterisk (*) originates from an impurity in the sample.



dodecamer is palindromic, only two resonances are observed. The resonance at 7.83 ppm originates from the adenine N6 proton, which forms the Watson-Crick hydrogen bond with O4 of thymine in each A · T basepair. The resonance at 5.90 ppm originates from the other proton in the adenine N6 amino group, which is not hydrogen-bonded. The linewidths of these resonances at 2.5°C are 54 and 47 Hz, respectively.

Representative spectra in ^{15}N -edited magnetization transfer experiments are shown in Fig. 2. As seen in the figure, with inversion of the resonance of a given amino proton, the intensity of the other proton in the amino group decreases because of cross-relaxation and exchange of the two protons via rotation of the amino group around the C-N bond. The time dependence of amino proton magnetizations in ^{15}N -edited magnetization transfer experiments is illustrated in Fig. 3. Four curves are obtained in each measurement, corresponding to each inverted proton and to the proton receiving the transfer of magnetization. Clearly, the two exponentials predicted by Eqs. 2 can be easily resolved: the fast component, $\exp(\lambda_2 t)$, dominates the time dependence for magnetization transfer times shorter than 50 ms (*lower panel* in Fig. 3), whereas the slow component, $\exp(\lambda_1 t)$, accounts for the evolution of the magnetizations for transfer times longer than 100 ms. It is interesting to note that the time dependence of the magnetizations for long magnetization transfer times depends on which amino proton is inverted (*upper panel* in Fig. 3). This effect originates from the fact that, because of the overlap of adenine amino proton resonances with other aromatic proton resonances (Fig. 1), the inverting pulse perturbs the magnetization of other DNA protons. For example, inversion of the non-hydrogen-bonded amino proton resonance at 5.90 ppm affects the resonances of deoxyribose H1' protons and those of non-hydrogen-bonded amino protons of cytosines. Being located more than 5 Å away, these protons do not influence relaxation of adenine amino protons of interest. On the other hand, inversion of the hydrogen-bonded adenine amino proton resonance at 7.83 ppm affects the magnetizations of H8 protons of adenine and guanines. These protons are located ~4–4.5 Å from the adenine amino protons. Their perturbation should not affect the relaxation of adenine amino protons for short magnetization transfer times, on which our determination of the rotation rate is based (Eqs. 3). However, at long times, inversion of these purine H8 protons should slow down the relaxation of adenine amino protons, as observed (Fig. 3).

The magnetization transfer experiments were carried out at 2.5 and 4°C, and the following values of the rotation rates of the adenine amino groups were obtained: $81 \pm 7 \text{ s}^{-1}$ at 2.5°C and $90 \pm 11 \text{ s}^{-1}$ at 4°C. At higher temperatures, the measurement of the rotation rates in ^{15}N -edited magnetization transfer experiments is limited by the fast relaxation and rotational exchange of amino protons during the selective pulse used for inversion. This effect is evident in Figs. 2 and 3 where, because of relaxation/exchange losses during the pulse, the intensities of the amino proton resonances at

short times after inversion are 15–25% of the equilibrium intensities. As the temperature is raised, this limitation becomes more severe due to the increase in the rotation rate. Because the length of the pulse should maintain the selectivity of inversion, magnetization losses during the inverting pulse cannot be reduced by shortening the pulse. Given these limitations, at higher temperatures, we have measured the rotation rates from lineshape analysis of ^{15}N -edited amino proton resonances.

For temperatures lower than 15°C, rotation of the adenine amino groups is slow on the chemical shift scale, and distinct resonances are observed for the two protons (Fig. 4). As the temperature is raised, the rotation rate increases and approaches the regime in which the rotational exchange is intermediate on the chemical shift scale. Because of this effect, in the temperature range from 15 to 55°C, the resonances are too broad for reliable measurements of linewidth (spectra not shown). Finally, at temperatures higher than 55°C, rotation is fast on the chemical shift scale, and a single, averaged resonance is observed for the two protons. It should be noted that, at the highest temperature investigated (i.e., 70°C), the dodecamer is well below its melting temperature (i.e., 83°C, as determined from the temperature dependence of nonexchangeable proton resonances), and thus dissociation of the duplex into single strands is negligible.

The linewidths of the amino proton resonances, $\Delta\nu_{1/2}^{A,B}$, should depend on the rate of rotation of the amino group, k_r , as well as on the rate of exchange of the amino protons with water, k_{ex} (e.g., see Eq. 4). To distinguish between these two effects, we have measured the rates of exchange of the amino protons with water in separate experiments of transfer of magnetization from water over the temperature range from 2 to 10°C. As shown in Fig. 5, no significant changes in the intensities of amino proton resonances were observed within 3 s after inversion of water resonance. This finding indicates that, at low temperatures and under the solvent conditions used, the exchange of adenine amino protons is too slow to be detectable by transfer of magnetization from water. Numerical simulations of the experiments gave a value of 0.04 s^{-1} as the upper limit for the exchange rates at low temperatures. This value is in agreement with the rates of solvent exchange of adenine amino protons measured by column chromatography in poly(A) · poly(U) and poly [d(AT)] · poly[d(TA)] (i.e., 0.012 and 0.009 s^{-1} , respectively, at 0°C; Teitelbaum and Englander, 1975). At higher temperatures, these rates are expected to increase. Previous investigations of free adenosine and related compounds have determined an energy of activation for the exchange of adenine amino protons with water of 10.5 kcal/mol at pH 7.0 (Cross et al., 1975). Using this value, we estimated that, at the highest temperature investigated, the exchange rates of adenine amino protons would be, at most, 2.1 s^{-1} . This value is much smaller than the observed linewidth (e.g., 74 Hz at 70°C), indicating that the contribution of the exchange with water to the linewidth can be safely neglected.

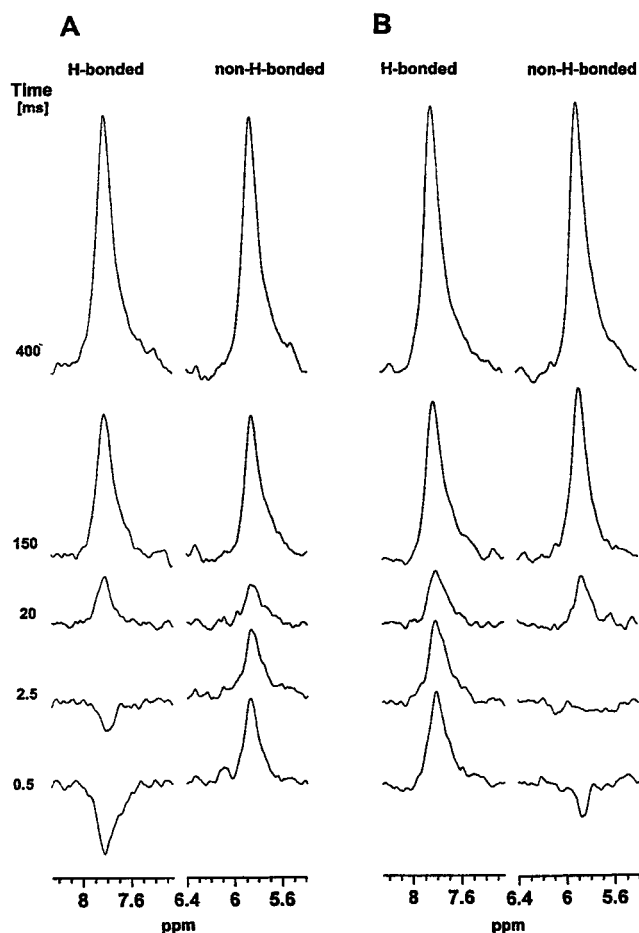


FIGURE 2 ^{15}N -edited magnetization transfer experiments at 2.5°C . The adenine amino proton resonances are shown at several values of the magnetization transfer time after inversion of the hydrogen-bonded (A) and non-hydrogen-bonded (B) amino proton resonance.

The rotation rates as a function of temperature are shown in Fig. 6. Also shown in the figure is the fit of the observed rates to the Eyring equation (Glasstone et al., 1941):

$$\ln\left(\frac{k_r}{T}\right) = \ln\left(\frac{k_B \cdot \kappa}{h}\right) - \frac{\Delta H^\ddagger}{RT} + \frac{\Delta S^\ddagger}{R} \quad (6)$$

where ΔH^\ddagger and ΔS^\ddagger are the activation enthalpy and entropy for rotation, respectively, k_B is Boltzmann's constant, R is the gas constant, h is Planck's constant, T is the absolute temperature, and κ is the transmission coefficient (assumed to be 1). The activation parameters obtained from the fit are $\Delta H^\ddagger = 15.3 \pm 0.2$ kcal/mol and $\Delta S^\ddagger = 6.0 \pm 0.7$ cal/(mol · K) (corresponding standard free energy change for formation of the activated state $\Delta G^\ddagger = 13.5 \pm 0.4$ kcal/mol). The errors in the activation parameters represent standard deviations in the linear fit.

DISCUSSION

The work presented in this paper provides the first determination of the rate and activation parameters for rotation of

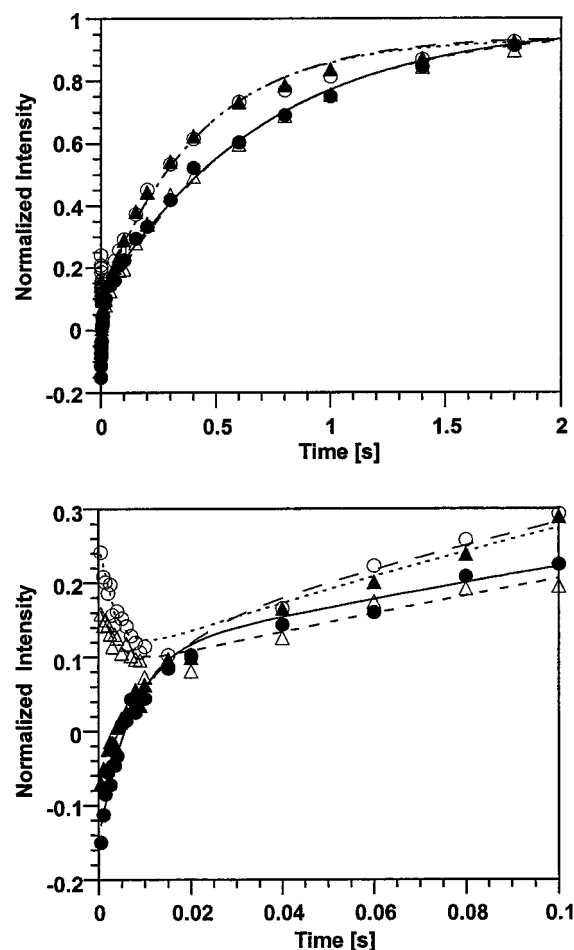


FIGURE 3 Dependence of the intensities of adenine amino proton resonances on magnetization transfer time at 2.5°C . \circ , \bullet , Hydrogen-bonded proton; \triangle , \blacktriangle , non-hydrogen-bonded proton; \bullet , \blacktriangle , intensity of the proton resonance inverted; \circ , \triangle , intensity of the resonance of the other proton in the amino group. The curves correspond to nonlinear least-squares fits to Eqs. 2. The lower panel shows an expansion of the plot for short magnetization transfer times.

the N6 amino group of adenine in a DNA double helix. The rotation rate varies from 60 to $24,000\text{ s}^{-1}$ over the temperature range from 0 to 70°C . At biologically relevant temperatures, for example, between 25 and 37°C , the rotation rates range from 700 to 2000 s^{-1} . These values are smaller than, or comparable to, the difference in resonance frequency between the two amino protons (e.g., $2\pi \cdot \delta\nu = 6100\text{ s}^{-1}$ for the dodecamer investigated at 500 MHz); hence, rotation of the amino group is in the range of the slow exchange regime just below the coalescence temperature. Under these conditions, the rotation rate is added to the natural linewidth (Eq. 4), explaining why, at these temperatures, the adenine amino proton resonances are broad and difficult to detect.

Internal rotation of exocyclic amino groups of the bases in isolated monomers as well as in nucleic acid structures is restricted by the partial double-bond character of the C-N bond. For free adenine, theoretical studies have estimated

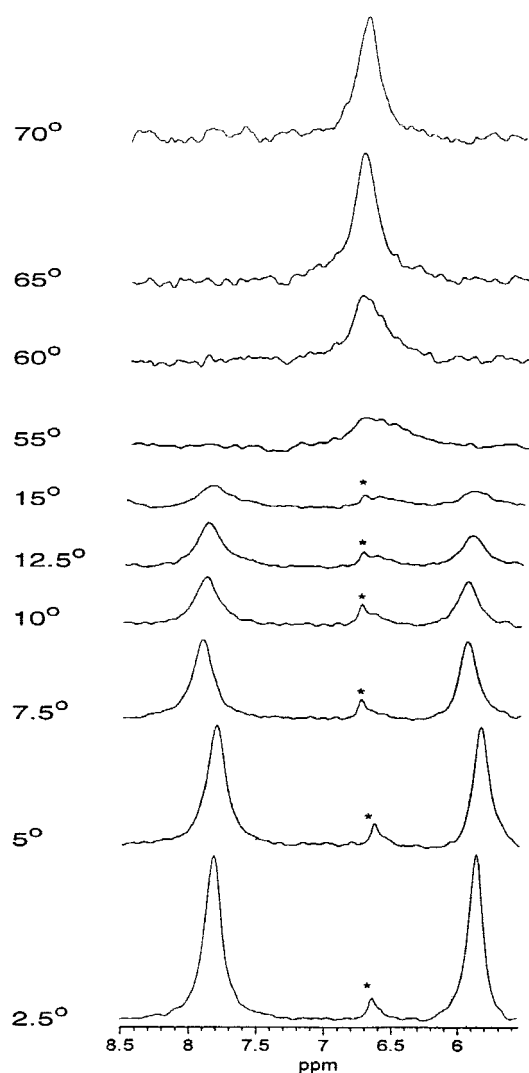


FIGURE 4 Temperature dependence of the ^{15}N -edited adenine amino proton resonances of the dodecamer.

the energetic barrier to the rotation of the amino group to be between 12 and 16 kcal/mol (Rao and Rao, 1973). This range encompasses the activation enthalpy for rotation, $\Delta H^{\ddagger} = 15.3 \pm 0.2$ kcal/mol, obtained in the present work. Experimental data for rotation of the amino group in adenine monomers are, at present, qualitative and scarce. In an early investigation of adenine amino protons in adenosine, AMP, and related compounds (Raszka, 1974), a single, relatively sharp resonance was observed for the two amino protons at temperatures as low as 0°C ; this indicates that, in free adenine monomers, rotation of the amino group is fast on the NMR chemical shift scale (e.g., rotation rate $\gg 200 \text{ s}^{-1}$ at 0°C ; Raszka, 1974). In contrast, our present results show that, in DNA, the rotation rate at the same temperature is slow, i.e., 60 s^{-1} . Hence, as expected, incorporation of the adenine into an A · T basepair of the double helix significantly lowers the rate of rotation of its amino group. One factor that could contribute to this effect is the change in the electronic structure of adenine upon basepairing. According

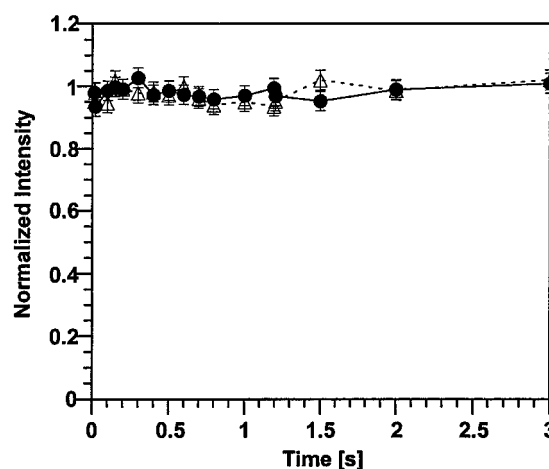


FIGURE 5 Normalized intensities of adenine amino proton resonances of the dodecamer in an experiment of transfer of magnetization from water at 10°C . ●, Hydrogen-bonded proton; △, non-hydrogen-bonded proton.

to quantum mechanical calculations, formation of the A · T base pair lowers the charge on the adenine amino nitrogen from 1.810 to 1.763 and increases the C6-N6 bond order from 0.454 to 0.506 (Pullman and Pullman, 1963). This enhancement of the double-bond character of the C6-N6 bond should raise the energetic barrier for amino group rotation in the basepair as compared to the free base.

Rotation of amino groups in double-helical nucleic acid structures should also perturb the Watson-Crick hydrogen bonds in which these groups participate. A possible molecular mechanism for this process has been proposed by Shaw and co-workers (Williams et al., 1990) for amino groups of cytosine and guanine in a model G · C basepair. Shaw and co-workers have found that rotation of the guanine N2 amino group in this isolated G · C basepair in deuteriochloro-

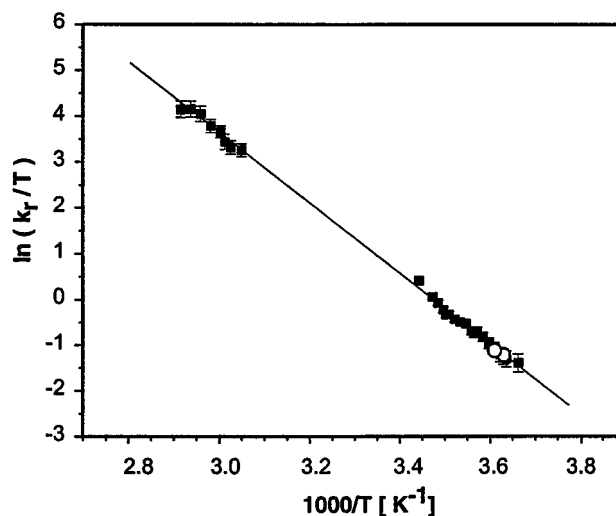


FIGURE 6 Temperature dependence of the rotation rates of the adenine amino group. ○, Values obtained from ^{15}N -edited magnetization transfer experiments; ■, values obtained from lineshape analysis. The line corresponds to the linear fit to the Eyring equation (Eq. 6).

reform has an activation enthalpy of 10.6 ± 0.3 kcal/mol and an activation entropy of -2.5 ± 1.4 cal/(mol \cdot K). In contrast, rotation of the N4 amino group of cytosine has an activation enthalpy of 18.6 ± 1.3 kcal/mol and an activation entropy of 11.2 ± 3.5 cal/(mol \cdot K). Based on these results, Shaw and co-workers proposed that rotation of the amino group in guanine occurs within the paired state of the base, whereas that of the amino group in cytosine occurs during transient opening of the G \cdot C basepair. We have evaluated this model for the case of the A \cdot T basepair studied in the present work. If rotation of the amino group occurs in the open state of the basepair, the observed rotation rate should depend on the rates of opening and closing of the basepair, k_{op} and k_{cl} , respectively. When the rate of closing is much larger than the rate of opening, this dependence can be expressed as

$$k_r = \frac{k_{op} \cdot k_r^0}{k_{cl} + k_r^0} \quad (7)$$

where k_r^0 is the rate of rotation of the amino group in the open state of the basepair. The rates of opening and closing of the A \cdot T basepair can be estimated from the rates of exchange of the imino proton in the basepair with water under the assumption that the open state required for rotation of the adenine amino group is the same as the open state involved in exchange of the thymine imino proton with water. Previous results from our laboratory (Folta-Stogniew et al., 1995) have shown that, in the DNA dodecamer of interest here at 20°C, the opening rate of the A \cdot T basepair, k_{op} , is $\sim 10^3$ s $^{-1}$, and the equilibrium constant for formation of the open state, K_{op} , is 1.7×10^{-5} ($K_{op} = k_{op}/k_{cl}$, and corresponding k_{cl} is 6×10^7). At the same temperature, the rotation rate of the adenine amino group predicted from Eq. 6 is 450 s $^{-1}$. Hence, in this case, rotation of the amino group is not rate-limited by the opening of the basepair. Moreover, the values of k_{op} and k_{cl} predict that the rate of rotation in the open state, k_r^0 , should be on the order of 5×10^7 s $^{-1}$. This value is several orders of magnitude higher than the rates of restricted rotation generally observed for amino groups in model purine compounds at ambient temperature (Oki, 1985). This observation suggests that rotation of the amino group occurs in a state close to that of the intact base pair and disturbs only the Watson-Crick hydrogen bond at the N6 position. The enthalpic cost for breaking this Watson-Crick hydrogen bond in DNA has been estimated to be 1.9 ± 1.1 kcal/mol (Williams et al., 1990); thus perturbation of interbase hydrogen bonding during rotation of adenine amino groups should contribute less than 15% to the overall activation enthalpy for rotation. Other factors, such as base stacking, local conformation of the amino group, and DNA hydration at or near the N6 position, should also affect the rotation of adenine amino groups in DNA. We are currently involved in efforts to identify these factors and their possible dependence on base sequence.

The rotation rates of adenine amino groups measured in this paper provide a quantitative description of one of the

conformational fluctuations present in a DNA double helix. This information will be significant for future structural studies of nucleic acids. NOE distance constraints to amino protons can currently be measured and used in structure refinement (Louis et al., 1998; Mueller et al., 1995; Zimmer and Crothers, 1995). Incorporation of the rotation of the amino groups in the dynamic analysis of NOE data is expected to enhance the accuracy of distance constraints to amino protons and thus improve the quality of NMR-derived structures of nucleic acids.

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